Ubiquitinated Protein Capture Kit

Item No. 15979

Customer Service 800.364.9897 *** Technical Support** 888.526.5351

www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600164	Ubiquitin Matrix (50% slurry)	1 vial/400 µl	4°C
600148	Biospin columns	20 tubes	4°C
600149	Collection tubes	20 tubes	4°C
600165	Ubiquitin Monoclonal Antibody (FK2) (HRP)	1 vial/25 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.
For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Microfuge tubes (1.5 ml)
2. Lysis Buffer - 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% (v/v) NP-40, 1 mM DTT, and protease inhibitor
3. Wash Buffer - 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% (v/v) NP-40, 1 mM DTT
4. Elution Buffer (as appropriate):
   - SDS-PAGE sample loading buffer - Western blot analysis
   - 0.1% formic acid solution - proteomic analysis
5. Target protein specific antibodies - Western blot analysis
6. Reagents for Western blotting
7. *Optional* - proteasome inhibitor, *e.g.*, MG132 (Cayman Item No. 10012628) or epoxomicin (Cayman Item No. 10007806)
8. *Optional* - DUB inhibitor, *e.g.*, ubiquitin-aldehyde

INTRODUCTION

Background

The covalent attachment of ubiquitin to proteins (ubiquitination) plays a fundamental role in the regulation of cellular function through biological events including cell cycle, differentiation, immune responses, DNA repair, chromatin structure, transcription, signal transduction, endocytosis, apoptosis, proteasomal protein degradation, and autophagy.[1-5] As such, ubiquitin signaling and the processes it mediates are essential for the normal functioning of cells and its dysfunction has been implicated in wide range of diseases including cancer, neurodegeneration, cardiovascular disease, and metabolic disorders.[1,3]

The type of ubiquitin modification, (monoubiquitin, multiubiquitin, polyubiquitin), substrate protein lysine residue(s) modified and, in the case of polyubiquitination, the chain length and lysine linkage type control the function and fate of ubiquitinated proteins.[1,3,5] In addition, all ubiquitin mediated pathways also utilize specific ubiquitin receptors to facilitate their regulation.[3,5]

Ubiquitination is achieved through three enzymatic steps.[4,5] In an ATP-dependent process, the ubiquitin E_1 activating enzyme catalyzes the formation of a reactive thioester bond with ubiquitin, followed by its subsequent transfer to the active site cysteine of a ubiquitin E_2 carrier protein. The selectivity of the ubiquitin cascade for a particular substrate protein relies on the interaction between the E_2 conjugating enzyme (of which a cell contains relatively few) and an ubiquitin E_3 ligase, of which over 600 have been identified to date. The specific E_2-E_3 pair required for ubiquitination of a particular substrate protein *in vivo* may also control the type, point, and length/linkage (polyubiquitin) of the ubiquitin modification.

About This Assay

Cayman's Ubiquitinated Protein Capture Kit facilitates the fast, effective capture and detection of ubiquitinated proteins from biological samples. The kit utilizes a high capacity, high specificity ubiquitin binding matrix together with an easy-to-use spin purification system for less 'hands on time' and superior performance. It allows purification of mono- and poly-ubiquitinated proteins, independent of chain linkage or length, but not free ubiquitin. The purification system is highly adaptable and compatible with samples from a wide range of species and with a broad range of lysis buffers. Analysis by Western blotting or proteomic methods enables identification and assessment of ubiquitinated proteins of interest. Each kit contains sufficient Ubiquitin Matrix to perform up to 20 assays.

This kit can be used to:

1. Capture and detect ubiquitinated proteins and free chains from cell lysates and tissue extracts.
2. Demonstrate specific proteins are substrates for ubiquitin modification *in vivo*.
3. Identify and characterize ubiquitin-modified proteins by proteomic analysis.
4. Investigate the role of ubiquitin in particular signaling pathways.

PRE-ASSAY PREPARATION

Assay Preparation

1. Sample Preparation

We recommend using 100-200 µl sample/control lysate at 5 mg/ml per assay as a starting point. Adjust the lysate concentration with Lysis Buffer if required.

2. Lysis Buffer Preparation

- This assay is compatible with a wide range of lysis buffers.
- Avoid buffer components that cause protein denaturation, especially chaotropes such as urea.
- Minimize use of reducing agents (*e.g.*, DTT) and detergents where possible.
- Suggested Lysis Buffer: 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 0.5% (v/v) NP-40, 1 mM DTT, and protease inhibitor.
- *Optional* - Include proteasome or DUB inhibitors in Lysis Buffer.

3. Elution Buffer Preparation

Select appropriate elution buffer for intended method of analysis.

- Western blot analysis - SDS-PAGE sample loading buffer
- Proteomic analysis - 0.1% formic acid

Assay Notes

Control reactions (recommended)

Widely used cell lysates such as HeLa S100 cytosolic fraction can be used as positive controls to demonstrate binding assay working/components functional.

Assay Optimization

Optimal assay conditions for capture of ubiquitinated proteins from specific lysate samples must be determined by the user. Adjustment of the following parameters may facilitate this process:

- Sample volume: 100-500 µl
- Sample concentration: 1-5 mg/ml
- Ubiquitin Matrix volume: 10-20 µl settled resin
- Assay time: 1-4 hours or overnight

ASSAY PROTOCOL

Hints

- Keep reaction components on ice throughout set-up.
- Save an aliquot of starting sample for later analysis.
- Include appropriate controls as required.

Ubiquitin Matrix Preparation

1. Resuspend the Ubiquitin Matrix by gentle inversion of the tube.
2. Aliquot 20 µl Ubiquitin Matrix suspension into required number of capped Biospin Columns.
3. Add 500 µl Wash Buffer to capped column.
   a. Mix for one minute.
   b. Remove base cap.
   c. Centrifuge at low speed (1,000-5,000 x g, one minute) to collect matrix.
   d. Discard flow through.
4. Repeat matrix wash/collection at least twice.

Ubiquitin Assay

5. Add 100-200 µl sample/control lysate to capped Ubiquitin Matrix column and mix by inversion.
6. Incubate for one hour at 4°C with rotary mixing.
7. Uncap column base and place in a collection tube.
8. Centrifuge at low speed (1,000-5,000 x g, one minute) to collect matrix.
9. Remove flow through and retain as 'Unbound Fraction' for subsequent analysis if required.
10. Replace column in collection tube.
11. Wash matrix by adding 500 µl Wash Buffer to column.
    a. Centrifuge at low speed (1,000-5,000 x g, one minute) to collect matrix.
    b. Repeat twice.

Elution of Captured Proteins

12. For SDS-PAGE/Western blot analysis:

a. Add SDS-PAGE sample loading buffer to capped column and mix by inversion.
b. Place column in microfuge tube.
c. Heat to 95°C for five minutes.
d. Remove base cap.
e. Centrifuge at low speed (1,000-5,000 x g, one minute) to collect eluted materials.
f. Analyse or store at -20°C.

NOTE: If required, pierce lid of spin tube prior to heating to prevent build-up of pressure.

13. For proteomic analysis:

a. Add 10 volumes (100 µl) 0.1% formic acid to capped column.
b. Rotary mix for 5-10 minutes at room temperature.
c. Uncap column base and place in microfuge tube.
d. Centrifuge at low speed (1,000-5,000 x g, one minute) to collect eluted materials.
e. Elution fraction can then be lyophilized and resuspended in trypsin digestion or alternative buffer prior to subsequent processing/analysis, or stored at -20°C.

Western Blot Analysis

Variable	Recommendation
SDS-PAGE	10% Gel
Samples for analysis	Starting sample; unbound (optional); elution
Target protein specific antibody (user supplied)	Western blotting conditions must be determined by the user and the antibody applied in conjunction with an appropriate secondary antibody

Table 1. Suggestions for Western blot analysis.

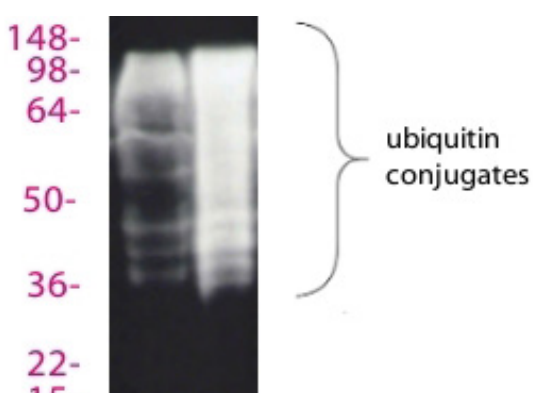

Figure 1. Western blot analysis of ubiquitin matrix capture of ubiquitin modified proteins from HeLa S100 lysate. Ubiquitin conjugates present in the starting sample (**1**) and Elution (**2**) samples were detected by Ubiquitin Monoclonal Antibody FK2 (HRP) at 1:1,000 dilution.

RESOURCES

References

1. Fulda, S., Rajalingam, K., and Dikic, I. Ubiquitylation in immune disorders and cancer: From molecular mechanisms to therapeutic implications. *EMBO Mol. Med.* **4(7)**, 545-556 (2012).
2. Shaid, S., Brandts, C.H., Serve, H., *et al.* Ubiquitination and selective autophagy. *Cell Death Differ.* **20(1)**, 21-30 (2013).
3. Husnjak, K. and Dikic, I. Ubiquitin-binding proteins: Decoders of ubiquitin-mediated cellular functions. *Annu. Rev. Biochem.* **81**, 291-322 (2012).
4. Komander, D. and Rape, M. The ubiquitin code. *Annu. Rev. Biochem.* **81**, 203-209 (2012).
5. Spasser, L. and Brik, A. Chemistry and biology of the ubiquitin signal. *Agnew. Chem. Int. Ed. Engl.* **51(28)**, 6840-6862 (2012).

Related Products

DUB Activity Assay Kit - Item No. 15981
Multiubiquitin Chain Monoclonal Antibody (Clone FK1) - Item No. 14219
Multiubiquitin Chain Monoclonal Antibody (Clone FK2) - Item No. 14220
Ubiquitin Interact Kit - Item No. 15978
Ubiquitin Monoclonal Antibody (Clone 5B9-B3) - Item No. 13722
Ubiquitin Monoclonal Antibody (Clone 6C11-B3) - Item No. 13723
Ubiquitin Polyclonal Antibody - Item No. 13724

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Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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